Applicability of recent methods used to estimate spontaneous baroreflex sensitivity to resting mice

Dominique Laude, Véronique Baudrie, and Jean-Luc Elghozi
Centre de Recherche des Cordeliers, Université Pierre et Marie Curie-Paris 6; Université Paris Descartes; and INSERM, U872, Paris, France

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Laude D, Baudrie V, Elghozi J-L. Applicability of recent methods used to estimate spontaneous baroreflex sensitivity to resting mice. Am J Physiol Regul Integr Comp Physiol 294: R142–R150, 2008. First published November 7, 2007; doi:10.1152/ajpregu.00319.2007.— Short-term blood pressure (BP) variability is limited by the arterial baroreflex. Methods for measuring the spontaneous baroreflex sensitivity (BRS) aim to quantify the gain of the transfer function between BP and pulse interval (PI) or the slope of the linear relationship between parallel BP and PI changes. These frequency-domain (spectral) and time-domain (sequence) techniques were tested in conscious mice equipped with telemetric devices. The autonomic relevance of these indexes was evaluated using pharmacological blockades. The significant changes of the spectral bandwidths resulting from the autonomic blockades were used to identify the low-frequency (LF) and high-frequency (HF) zones of interest. The LF gain was \( 1.45 \pm 0.14 \) ms/mmHg, with a PI delay of 0.5 s. For the HF gain, the average values were \( 2.0 \pm 0.19 \) ms/mmHg, with a null phase. LF and HF bands were markedly affected by atropine. On the same 51.2-s segments used for cross-spectral analysis, an average number of 26.4 ± 2.2 slopes were detected, and the average slope in resting mice was \( 4.4 \pm 0.5 \) ms/mmHg. Atropine significantly reduced the slopes of the sequence method. BRS measurements obtained using the sequence technique were highly correlated to the spectral estimates. This study demonstrates the applicability of the recent methods used to estimate spontaneous BRS in mice. There was a vagal predominance in the baroreflex control of heart rate in conscious mice in the present conditions.

Baroreceptors; heart rate; sympathetic; vagus nerve

THE IMPORTANCE OF THE BAROREFLEX in blood pressure (BP) regulation in mice can be appreciated by the marked increase in BP variability that occurs after sinoaortic deafferentation of baroreceptors that are located in the carotid sinuses and aortic arch (9, 21). The ability of the baroreflex to buffer BP fluctuations varies depending on the frequency of the BP fluctuations (19). BP and heart rate (HR) fluctuate at regular frequencies, the magnitude of which can be accurately quantified using power spectral analysis (25, 30). The absolute frequency bands at which these oscillations occur in mice were analyzed in a previous report (3). One recent approach to estimating the spontaneous baroreflex sensitivity (BRS) over a stationary period is the calculation of the gain of the transfer function between BP and R-R interval in the low-frequency (LF) and high-frequency (HF) bands (20, 28). This spectral analysis approach has been recently applied to conscious mice (2, 7, 9–12, 16, 18, 32). Another recent approach is the dynamic evaluation of the spontaneous BRS called the sequence technique (4). This method is based on the computerized scanning of beat-to-beat series of systolic BP and R-R interval values in search of spontaneous sequences of consecutive increases or decreases in systolic BP associated with parallel changes in R-R intervals indicating baroreflex responses. The sequence technique has also been recently applied to conscious mice, with different parameters (length of the sequences, threshold for BP and RR changes, threshold for the coefficient of correlation between systolic BP and R-R interval changes, delay in heartbeats between systolic BP and RR) (2, 7, 10, 21, 34). The traditional methods used to estimate BRS mainly refer to the invasive approaches such as the study of reflex HR (or R-R) changes resulting from BP increases or decreases resulting from the administration of vasoactive drugs (phenylephrine or sodium nitroprusside) with various protocols applied to conscious mice by many authors for > 10 yr (24).

The values derived from the recent procedures of spontaneous BRS estimation have been repeatedly compared with the values obtained with the traditional invasive approach that induces pharmacological changes in BP. Differences in the meaning of these complementary methods are still a matter of debate (27).

The discrepant estimations of spontaneous BRS in resting mice, varying from 1 to 4 ms/mmHg in the studies cited above, may reflect different hemodynamic states that largely depend on the experimental conditions (15, 31). Two determinant factors are 1) the BP measurement procedure, which may be an externalized catheter, or telemetric recordings allowing the animals to freely move; and 2) the delay of recovery from the surgical procedure, usually too short in the case of surgery to implant an externalized catheter. In addition, a limiting factor for studying mice is the natural occurrence of frequent and rapid changes in BP and HR, which limit the length of stationary periods used for BRS estimations with spectral analysis (14). Finally, the spectral bandwidths used to estimate the spontaneous BRS by the spectral method differ among the authors and this may also lead to different BRS estimates.

In the present study, the spontaneous BRS was evaluated in conscious mice equipped with telemetric devices, using one frequency-domain technique [spectral method based on the transfer function between BP and pulse interval (PI)] and one time-domain technique [sequence method]. Autonomic pharmacological blockade with conventional drugs (atropine, prazosin, atenolol) was used to assess the sympathetic and vagal influences on the spontaneous BRS and to identify the spectral zones of autonomic relevance. The effect of hydral-
azide was also tested to detect the effects of sympathetic activation resulting reflexly from the vasodilatory action of the drug. To test the hypothesis that the spontaneous beat-by-beat interactions of systolic BP and PI reflected true baroreflex events, rather chance interactions, we also assessed the methodology of spontaneous BRS (sequence method and cross-spectral analysis) by surrogate data analysis (5).

MATERIAL AND METHODS

The protocol has previously been published (3) but is outlined below. Experiments were performed in eight adult male mice (C57BL/6; Charles River, L’Arbresle, France; 28 ± 2 g) in accordance with the relevant guidelines of the French Ministry of Agriculture for scientific experimentation on animals and with European Communities Council Directive. Our personnel are authorized to conduct such investigations according to the Ministry’s Executive Order No. 75–215.

Surgery

Mice were anesthetized initially with 5% isoflurane in an oxygen stream and maintained on 2–3% isoflurane. Mice were kept on a heating pad throughout implantation of the BP telemeter (model TA11PA-C10; Data Sciences International, St. Paul, MN). The catheter was inserted into the left common carotid artery with the tip in the aortic arch, and the telemetric transmitter probe was positioned subcutaneously on the right flank (6). This procedure was preferred to aortic catheterization to minimize the surgical procedure and then facilitate the recovery process, although it suppressed the left internal carotid discharges, which may, at least temporarily (~1 wk in rats (26)), influence the baroreflex function. To reduce any infection and pain, the mice received one dose (20 mg/kg ip) of amoxicillin (Clamoxyl; SmithKline Beecham, Nanterre, France) and one dose (5 mg/kg ip) of ketoprofen (Profenid; Aventis, Paris, France). After the mice had recovered from the anesthesia in a warm (36°C) box, they were housed in individual cages placed on top of the telemetric receivers in a light-dark cycled recording room, for a 2-wk recovery period before the initiation of the experiment. This period was provided to allow recovery of the animal, and to condition the animals to the recording room. Mice were fed standard A04 mouse chow (Scientific Animal Food and Engineering, Augy, France) with water ad libitum.

Experimental Protocols

Mice were randomly assigned to one of the four following treatment sessions: saline (4 ml/kg), atropine methyl nitrate (2 mg/kg, 4 ml/kg; Sigma, St. Louis, MO), atenolol (1 mg/kg, 4 ml/kg; Sigma), prazosin (1 mg/kg, 4 ml/kg; Sigma), or hydralazine (0.2 mg/kg, 4 ml/kg; Sigma). A washout period of 48 or 72 h separated two sessions. Recordings were obtained during the morning when the mice were generally quiet. Each session included a control 30-min recording, an intraperitoneal injection, and a posttreatment 30-min recording initiated 30 min after the intraperitoneal injection. This interval allowed recovery from the stress associated with handling and injection.

Doses of the autonomic blockers were chosen after separate preliminary validation experiments had been performed. This validation procedure compared the effects of agonists (methacholine, phenylephrine, isoprenaline) on HR and BP levels before and after the administration of selected doses of the blockers (atropine, prazosin, atenolol).

Telemetry Data Acquisition

The telemetered BP signal obtained after the magnetic activation of the probe was generated as a calibrated analog signal (UA10; Data Sciences International) with a range of ±5 volts. This signal was then digitized at a rate of 1,000 Hz and processed by an algorithm based on feature extraction to detect and measure the characteristics of BP cycles (Notocord-hem, version 3.2; Notocord Systems, Croissy sur Seine, France). Using the acquisition software, the experimental data were recorded continuously in real time and stored on the local hard disk. Systolic BP was derived from the aortic BP waveforms sampled at 1,000 Hz on a beat-by-beat basis. These beat-by-beat systolic BP time series were linearly interpolated to generate a new time series for systolic BP that was sampled at an equidistant sampling interval of 0.05 s (sampling rate of 20 Hz). PI (surrogate for R-R interval) was calculated as the time period between two successive temporal derivative of the BP (dBP/dtmax) points and stored with the systolic BP associated with the first dBP/dtmax. A linear interpolation was used to convert nonequidistant beat-by-beat time series to equidistant time series sampled at 20 Hz.

Individual data were exported from the acquisition software and transferred to a Microsoft Excel 97 spreadsheet for calculations. Each recording was visualized to select one period without erratic fluctuations of enough duration (>51.2 s) for each session, which would be used for the BP and PI variability study. An example of a long recording, including a zoom on the selected period, was shown in the previous paper (3). The estimates of spontaneous BRS were calculated on the same segments as those used for power spectral analysis in that paper (3).

Spontaneous BRS Estimations

Spectral method. The rhythmicities of BP and PI were evaluated by power spectral analysis using a fast-Fourier transformation. The BP signal processing and spectral analysis were modified from those previously described in rats (17). Briefly, the evenly spaced sampling allowed direct spectral analysis using a fast-Fourier transform algorithm of a 1,024-point series, corresponding to a 51.2-s period. Each spectral component band was a harmonic of 20/1,024 Hz, i.e., 0.019 Hz. The gain of the transfer function between systolic BP and PI intervals used to estimate the spontaneous BRS (ms/mmHg) was calculated in the previously identified LF (0.15–0.60 Hz) and HF (2.5–5 Hz) bands (3).

In addition, a validation procedure of the optimal frequency bands used for spontaneous BRS estimates was applied to the gain of the transfer function, according to previous reports (3, 13). The analysis of the effect of atropine on the gain (average changes or significance of the changes) was calculated for all the combinations of bandwidths of the spectrogram, from one frequency band resolution to the entire spectrum, to detect the zones responding to autonomic drugs.

Sequence method. Ramps of progressive increases or decreases in systolic BP or PI were automatically detected in the beat-by-beat time series. Sequences defined ramps of three or more systolic BP value associated with parallel PI ramps, with up sequences associating systolic BP increases and PI lengthenings and down sequences associating BP decreases to PI shortenings. A large number of up and down sequences occurred during the 51.2-s segments used for the analysis, with an average number of 26.4 ± 2.2 (n = 40 segments). An example of systolic BP and PI recordings identifying the up and down sequences is shown in Fig. 1. The spontaneous BRS was calculated as the slope (ms/mmHg) of the linear regression lines between the systolic BP and the subsequent PI intervals. The baroreflex effectiveness index (BEI), which provides information on the baroreflex function that is complementary to BRS was also calculated (8). It is defined as the ratio between the number of systolic BP ramps followed by the respective reflex (parallel) PI ramps (numerator), and the total number of systolic BP ramps observed in a given time window (denominator). To further characterize the optimal sequence method for estimating the spontaneous BRS in mice, we analyzed: 1) the length of the sequences; 2) the effect of introducing various delays of heartbeats between systolic BP and the reflex changes in PI (from 0 to 10); 3) the effect of introducing a threshold in BP (from 0 to 2 mmHg, by steps of 0.2 mmHg; 4) the
effect of introducing a threshold in PI (from 0 to 10 ms, by steps of 1 ms); and 5) the effect of introducing a threshold in the coefficient of correlation ($r$) (from 0 to 0.95 by steps of 0.05) between BP and PI.

**Statistical Analysis**

Results are expressed as means ± SE. A one-way ANOVA was used to estimate the influence of treatments on the estimates (gains for the spectral method or number of sequences, slopes, and BEI for the sequence method) of the spontaneous BRS, followed by a pairwise multiple comparison procedure (Fisher least significant differences method). When the variance ratios were significantly high, a logarithmic transformation was applied.

The relationship between the sequence method and the spectral approach estimates of spontaneous BRS was evaluated by linear regression.

The quantitative (average) changes in gain induced by atropine on the various combinations of frequency bands were analyzed as well as the significance of the changes induced by atropine. Paired t-tests were used to characterize the effect of atropine, and three levels of significance were used ($P < 0.05$, $P < 0.01$, and $P < 0.001$) to represent the effects of atropine on the different bands of the spectrum of the gain of the transfer function. Since the values of gain in adjacent frequency bands do not vary independently, a Bonferroni correction would have been too conservative. We therefore plot the $P$ values themselves below the conventional 0.05, 0.01, and 0.001 levels so that the degrees of significance are evident to the reader (1).

**RESULTS**

**Spectral Method**

We used the optimal LF (0.15–0.60 Hz) and HF (2.5–5.0 Hz) bands for detecting autonomic changes in resting mice (3), and performed cross-spectral analysis on 51.2-s segments. Average gain in the LF band using predrug recordings ($n = 40$) was 1.45 ± 0.14 ms/mmHg, with an average coherence of 0.37 ± 0.03 and a phase of −1.2 ± 0.11 rad corresponding to a PI delay of 0.5 s. For the HF gain, the average values were 2.0 ± 0.19 ms/mmHg, with an average coherence of 0.41 ±...
0.02 and a phase of 0.1 ± 0.1 rad, indicating that systolic BP and PI were in phase.

Effect of Drugs

The effects of drugs on the LF and HF gains are shown in Fig. 2. Atropine markedly reduced the LF and HF gains and also significantly reduced the coherence of the transfer function in the HF range (from 0.42 ± 0.04 to 0.29 ± 0.03, \( P < 0.001 \)).

Two representations were used to illustrate the spectral changes induced with atropine as shown in Fig. 3. The amplitude of the changes in gain was widely distributed with fewer changes in the higher frequencies. The qualitative picture showed zones of interest that were significantly affected by atropine. The significant changes do not match the average changes. Two zones of interest were clearly identified, with a LF zone below 0.63 Hz (\( P < 0.01 \)) and a HF zone between 2.7 and 5.0 Hz (\( P < 0.01 \)). The zone of

![Fig. 3. Average gain changes induced by atropine (left) and significance of these changes (right) obtained in the 8 mice. Abscissa and ordinates defined all the analyzed bandwidths (32,896) with the abscissa corresponding to the low bound and the ordinate the high bound of any given point. The degree of gain changes is represented by the scale of 6 colors from 0 to 3 ms/mmHg. The degree of significance is represented by the scale of 4 colors with nonsignificant (NS), \( *P < 0.05 \), \( **P < 0.01 \), \( ***P < 0.001 \). As an example, the frequency range 3.5–4.5 Hz is associated with a decrease in gain approximating 1 ms/mmHg (left), with \( P < 0.001 \) (right).](image-url)

![Fig. 4. Effects of changing the length of the sequences (from 3 to 8 beats) on the number of sequences, the slope, and the baroreflex effectiveness index (BEI) \( (n = 40) \). The up and down sequences are combined on the left. The up and down sequences are separated on the right \( (n, \text{number}) \).](image-url)
The highest significance within these two bands were 0–0.5 Hz ($P < 0.01$) and 3.7–4.0 Hz ($P < 0.001$).

**Sequence Method**

**Criteria.** First, the sequences were analyzed prior to drug treatments ($n = 40$ segments). Figure 4 summarizes these data. As a delay of three beats is close to the phase of 0.5 s derived from the cross-spectral analysis between BP and PI, the following sequence calculations were performed using a three-beat delay. It is noteworthy that the same calculations performed with a zero-beat delay did not consistently change the results.

**Length of the sequences.** Most sequences were made of three consecutive beats (25.6 ± 1.2). Few sequences of four beats were observed (0.6 ± 0.2). The number of sequences with five beats or more was negligible. The BEI represented about 27% of all ramps. The average number of up sequences was 16.7 and the number of down sequences was 9.9. Slopes obtained with the up and down sequences were not different (3.7 ± 0.31 vs. 5.4 ± 0.96 ms/mmHg for all the sequences).

**Effects of the Delay**

The effect of introducing a delay between BP and PI for considering and analyzing the sequences is shown in Fig. 5. The number of sequences, BEI, and gain vary cyclically with a period of three beats (about 0.44 s) and dampen with increased delays.

**Effects of a BP Threshold**

Thresholds from 0 to 2 mmHg (in steps of 0.2 mmHg) were applied to the BP changes for validating a BP ramp. A threshold of 0.2 mmHg reduced the number of sequences from 26.4 ± 2.2 to 22.7 ± 2.0 with a reduction of slope from 4.4 ± 0.5 to 3.7 ± 0.4 ms/mmHg. A threshold of 0.4 mmHg induced a further reduction in the number of slopes (to 18.6 ± 1.8) and a further reduction in slope (to 3.4 ± 0.3 ms/mmHg). Higher thresholds in BP dramatically decreased the number of sequences and the corresponding slopes.

**Effects of a PI Threshold**

Thresholds from 0 to 10, in steps of 1 ms, were applied to the PI changes for validating a sequence. A threshold of 1 ms reduced the number of sequences from 26.4 ± 2.2 to 16.4 ± 1.5 with an increase in slope from 4.4 ± 0.5 to 5.3 ± 0.7 ms/mmHg. A threshold of 2 ms induced a further reduction in the number of sequences (to 9.8 ± 1.1) and a further increase in slope (to 6.8 ± 1.1 ms/mmHg). Higher thresholds in PI led to a dramatic decrease in the number of sequences and BEI associated with an increase in slope.

![Fig. 5. Effects of introducing a delay from 0 to 12 beats between parallel systolic blood pressure and pulse intervals ramps on the number of sequences, slope, and BEI on original data (solid lines, $n = 40$) and randomized data (dotted line).](http://ajpregu.physiology.org/ by 10.220.32.247 on June 28, 2017)
Effects of a Coefficient Of Correlation Threshold

Finally, thresholds for the linear correlation coefficient were applied, from 0 to 0.95, (in steps of 0.05). There were negligible effects when $r$ varied from 0 to 0.70. However, a marked effect was seen when the threshold applied for $r$ was 0.90 or 0.95. The number of sequences decreased to 20.8 ± 1.9 for 0.90 and 15.8 ± 1.5 for 0.95 and the values of slopes were 3.8 ± 0.3 ms/mmHg when $r$ was fixed to 0.90 or 0.95. The BEI decreased to 0.21 ± 0.02 for 0.90 and 0.16 ± 0.01 for 0.95.

Data Summary

Altogether these observations led us to analyze sequences of three beats between systolic BP and PI ramps of similar direction (up or down sequences) and to combine up and down sequences, with a duration of three or more beats, with a delay of three beats between BP and PI, no threshold for systolic BP or PI changes, and no threshold for $r$. Using these criteria the average number of sequences detected in the 51.2-s window ($n = 40$, predrug recordings) was 26.4 ± 2.2, the average $r$ was 0.93 ± 0.01, the average slope was 4.4 ± 0.5 ms/mmHg, and the average BEI was 0.27 ± 0.02.

Effects of Drugs

Using the simple criteria defined above, the effect of drugs on the spontaneous BRS derived from the sequence method were computed and are shown in Fig. 6. The main effect of drugs was limited to atropine, which significantly reduced the slopes (to 0.8 ± 0.12 ms/mmHg). The increase in slope observed with prazosin did not reach significance.

Surrogate Data

Original systolic BP and PI data sets ($n = 40$) were randomized. The mean and variances of the randomized series were equal to those of the original time series. The randomized data were analyzed and compared with the original data by using the spectral and the sequence methods. The spectral method indicated small values of LF and HF gains with little coherence and a LF phase close to zero (LF gain 1.44 ± 0.33 ms/mmHg for original data vs. 0.79 ± 0.07 ms/mmHg for surrogate data, $P < 0.001$; HF gain 2.03 ± 0.19 ms/mmHg vs. 0.84 ± 0.07 ms/mmHg, $P < 0.001$; LF coherence 0.37 ± 0.02 vs. 0.13 ± 0.01, $P < 0.001$; HF coherence 0.41 ± 0.02 vs. 0.14 ± 0.01, $P < 0.001$; LF phase −1.22 ± 0.11 rad vs. −0.11 ± 0.11 rad; $P < 0.001$, $n = 40$). For the sequence method, randomized data showed a constant number of sequences (15.1 ± 0.15) with no effect of the delay and a constant slope of 3.3 ± 0.07 ms/mmHg. Original data exhibited a significantly higher number of sequences ($P < 0.001$), a higher BEI ($P < 0.001$), and a higher slope ($P < 0.01$) for the delays of zero and three beats, compared with surrogate data. The effects of introducing a delay between surrogate BP and PI values on the sequence analysis are shown in Fig. 5.
Comparison of the Two Methods

Reproducibility of the two methods was tested by comparing the estimates of the predrug sessions. No significant changes were observed between the sessions. The sequence method indicated higher estimates of spontaneous BRS compared with the LF and HF gain. Correlations between the LF gain or HF gain and the slope of the sequence method obtained in the different conditions (saline or drugs) are shown in Fig. 7. The slopes of the sequence method were significantly correlated to the LF gain \( (r = 0.85, P < 0.01) \) and the HF gain \( (r = 0.90, P < 0.001) \).

Coefficients of Variation

The within- and between-animal coefficients of variation (CV) were derived from the before-drug sessions. The mean within-animal CV (same mouse recorded at five different days) was 51.2%, 45.5%, and 50.1% for the LF gain, the HF gain, and the slope of the sequence method, respectively. The between-animal CVs were derived from the eight mice data recorded during the same session. These between-animal CVs were 57.7%, 61.4%, and 56.9% for the LF gain, the HF gain, and the slope of the sequence method, respectively. When the values of LF gain, HF gain, and the slope of the sequence method of the eight mice obtained in 1 day were averaged, CVs between days were 17.7%, 7.6%, and 4.1%, respectively.

![Diagram](http://example.com/diagram.png)

Fig. 7. Correlation between LF gain or HF gain and slope of the sequence methods obtained before (open symbols) and after (black symbols) saline (circle, \( n = 8 \)), atropine (diamond, \( n = 8 \)), hydralazine (hexagon, \( n = 8 \)), atenolol (square, \( n = 8 \)), or prazosin (triangle, \( n = 8 \)).

DISCUSSION

The main features of this study were the applicability of the recent time- and frequency-domain methods used to estimate the spontaneous BRS in mice equipped with a telemetric device for BP recording. Surrogate data analysis indicated that the spontaneous interactions of systolic BP and PI are not just random interactions. Slopes calculated using the sequence technique were highly correlated to the spectral estimates i.e., gains of the transfer function between BP and PI. A vagal predominance in the baroreflex control of HR was demonstrated by using atropine, which markedly attenuated the BRS values.

The BP telemetric recording is a technical improvement that has proven very useful in mice for detecting cardiovascular adaptations after genetic, pharmacological, environmental, or surgical modifications in this species. The development of miniature implantable radiotelemetric devices offers the possibility of long-term BP measurements in untethered rodents living in their home cages. The impact of surgery is considerable in these small animals, and the procedure also allows extension of the recovery period as required for physiological measurements (15). It was previously shown that HR levels were low (418 beats/min or 147 ms for PI) in the acclimatized mice analyzed in our study, recorded during a resting period (morning) by telemetry (3). The dominant role of the vagus in these conditions was translated into marked HR changes (HR level, LF and HF power of the PI spectra) after atropine, while the cardiac sympathetic control was minimal at rest, as shown by the minor effect of atenolol.

The sequence technique is particularly suitable to mice because it provides information on the dynamic aspects of baroreflex control of HR during spontaneous behavior, with roughly one value of slope relating BP to HR every 2 s. The present estimates of baroreflex function occurred under conditions where the baroreflex was not too far from its baseline operating point since spontaneous BP changes were of small amplitude (<10 mmHg) within the periods of observation. This operating point was probably located at the inflexion point of the sigmoid barocurve since up and down sequences indicated closed estimates (2).

Besides the experimental considerations, it has to be pointed out that the estimates of BRS presently obtained using the recent methods were close to the maximal gain derived from the full baroreflex curves (roughly between 2 and 5 ms/mmHg) (22–24, 29). The sequence technique (2, 7, 10, 21, 34) or the spectral method (gain of the transfer function or alpha coefficient) (2, 7, 9–12, 16, 18, 32) also indicated values of BRS in the same order of magnitude as the present values, between 1 and 4 ms/mmHg. The sequence method indicated estimates close to the HF gain and slightly higher than the LF gain, using the same samples. This difference in the estimates was even more marked in humans (20). The reason might be the vagal predominance in the rapid HR responses explored using the sequence method or the HF gain. In contrast, the LF gain may include the sluggish contribution of the heart sympathetic nerves. Such a reasoning formulated in other species, including man, may not be applied to our mice for two reasons. First, \( \beta \)-adrenergic blockade achieved with atenolol did not affect the LF gain of the transfer function, supposed to reflect the cardiac sympathetic contribution to the cardiac baroreflex function.
Second, atropine drastically reduced the LF gain (8% of the resting LF gain), a reduction more marked than the effects of atropine on the HF gain (31% of the resting HF gain) or on the slope of the sequence technique (20% of the resting slope). This illustrated the predominance of the vagus in the LF gain under our conditions. Interestingly, Tank et al. (32) showed in conscious mice that clonidine is able to increase 10-fold the average slope calculated by the sequence method and also the LF gain (transfer function), and these effects were abolished with atropine. These observations corroborate the role of the vagus in the baroreflex mechanisms, activated with clonidine. To our knowledge, there is only one report on the effects of autonomic blockers on spontaneous BRS indexes in conscious mice (29). Sakai et al. (29) analyzed the full barocurve using infusions of random doses of phenylephrine or nitroprusside. Vagal and β-adrenergic blockade (atropine and propranolol) affected to a similar degree (~50% reduction) the maximal gain of the baroreflex, indicating a mixed contribution of the two limbs of the autonomic system to the baroreflex control of HR evaluated using this procedure (29). It is noteworthy that the steady-state evaluation of baroreflex function allows the slow sympathetic control of HR to set up (33). The experimental conditions are also of paramount importance in the tuning of the sympathovagal balance. It is likely that the sympathetic contribution to the spontaneous BRS, negligible here, could be amplified in other conditions, such as those associated with high HR levels, often observed when BP catheters are used and when delays for recovery are short, as previously noted (3).

The functional value of these indexes to estimate BRS was illustrated by the effects of baroreceptor denervation, which was shown to markedly reduce the BRS indexes in conscious mice. Masuki et al. (22) and Martinka et al. (21) demonstrated that the spontaneous parallel fluctuations in BP and PI were markedly reduced after surgery. Fazan et al. (9) showed that sinoaortic deafferentation affected the spectral indexes of BRS, as well as the HR responses to changes in BP induced by phenylephrine and nitroprusside. In that study, there was a loss of coherence between LF fluctuations in systolic BP and PI after denervation. Findings that LF oscillations in systolic BP and PI are coherent in intact mice with changes in systolic BP leading the changes in PI [phase lag 0.6 s for Fazan et al. (9), 0.5 s in this study] suggest that the changes in PI are driven, at least in part, by changes in baroreceptor activity. The marked decrease in LF PI variability and loss of coherence between systolic BP and PI oscillations in the LF range after sinoaortic denervation strengthens the interpretation that this measure of spontaneous BRS is valid in mice (9). In contrast, although HF oscillations in systolic BP and PI oscillations are coherent, the absence of an appropriate phase lag between systolic BP and PI [null phase for Fazan et al. (9), null phase as well in this study] suggests that the changes in PI in this HF range are not mediated by the baroreflex (9). Nevertheless it has to be stressed that the phase cannot exceed 0.2 s for signals oscillating at 2.5 Hz. In other words the information given to the phase in this HF range must be tempered. Thus, there is no decisive argument to conclude whether or not the gain in the HF band represents a baroreflex mechanism. If the systolic BP and PI oscillations in the HF range are not functionally related, the vagal contribution to the HF PI fluctuation is likely, as demonstrated with atropine, and this may account for the reduced gain of the transfer function in the HF range. This reasoning may also be applied to the LF range, although more arguments support the concept that LF gain reflects baroreflex mechanisms.

The functional significance of the sequence technique was considered after applying a delay of 0.5 s (3 beats) between BP and PI changes, close to the physiological delay between these two variables (9, 22, 23, present study). This delay was associated with a high number of sequences and an efficient baroreflex function, estimated using the slope and the BEI. The number of sequences and BEI exhibited a cyclic pattern of three to four beats period, attributable to spontaneous oscillations of BP and PI. It is remarkable that a high number of sequences and a high BEI were also observed with a zero-beat delay (Fig. 5). Respiration, which corresponds to the HF oscillations of BP and PI within the HF range, may possibly influence the BRS. Indeed, a low-pass filtering did not influence the slope of the baroreflex but increased the number of sequences and the BEI (results not shown). This could indicate that respiration inhibits the baroreflex function, but further studies are necessary to investigate this issue.

**Perspectives and Significance**

The recent time- and frequency-domain methods used to estimate spontaneous BRS in mice are applicable to conscious resting mice equipped with a telemetric device for BP recording. The LF and HF zones for the cross-spectral analysis were superimposable on those reported in the previous paper (3) i.e., 0.15–0.60 Hz for the LF band and 2.5–5.0 Hz for the HF band. The sequence method can be used with a delay of zero or three beats. Thresholds for the coefficient of correlation, BP, or PI changes were unnecessary. All the estimates of spontaneous BRS were markedly affected by atropine, indicating that under the present conditions, the baroreflex function was predominantly under the influence of the vagal nerves, with no detectable cardiac sympathetic control. Additional experiments should be made in conditions associated with sympathetic activation to characterize to which extent the sympathetic may affect the baroreflex function. Future studies should apply the parameters of the present study to quantify the spontaneous BRS in mice.

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